

WHAT IS CLAIMED IS:

1. A process of fragmenting and labeling at least one synthetic or natural member selected from the group consisting of DNA and chimeric DNA-RNA polymers, comprising the steps of:

5 chemically fragmenting said member in the presence of at least one multivalent metal cation in a substantially aqueous solution to produce a plurality of fragments; and

attaching at least one label to at least one of said fragments with a labeling agent to produce a detectably labeled fragment in said aqueous solution.

10 2. The process according to claim 1, wherein said DNA comprises at least one thiophosphate nucleotide.

3. The process according to claim 1, wherein reagents used in the fragmenting and attaching steps are added to an *in vitro* nucleic acid amplification mixture.

15 4. A process of fragmenting and labeling a synthetic or natural nucleic acid, comprising the steps of:

20 chemically fragmenting the nucleic acid in the presence of at least one multivalent metal cation in a substantially aqueous solution to produce a plurality of fragments;

attaching at least one label to at least one of said fragments with a labeling agent to produce a detectably labeled fragment in said aqueous solution; and then

treating said aqueous solution to decrease or eliminate unattached labeling agent.

Sub
C1

09736151.121500

Sub
C2

5. The process according to claim 4, wherein the treating step comprises adding a quencher to the aqueous solution after the fragmenting and attaching steps.

6. The process according to claim 5, wherein the quencher is a pyrophosphate, thiol derivative, chelating agent, phosphate anion or carbonate anion.

5 7. The process according to claim 4, wherein the treating step physically separates the labeled nucleic acid fragment from unattached labeling agent in the aqueous solution after the fragmenting and attaching steps.

8. The process according to claim 7, wherein the treating step further includes adding an acid to the mixture after the fragmenting and attaching steps.

10 9. The process according to claim 7, wherein the treating step further includes adding a chelating agent to the mixture after the fragmenting and attaching steps.

15 10. The process according to claim 7, wherein the treating step uses an organic solvent to separate the labeled nucleic acid fragment from the unattached labeling agent.

11. The process according to claim 10, wherein the organic solvent is 1-butanol, 2-butanol, isopentyl alcohol, 1-pentanol or cyclohexanol.

20 12. The process according to claim 7, wherein the treating step separates the labeled nucleic acid fragment from the unattached labeling agent by using solid phase extraction of the nucleic acid fragments on a solid support.

13. The process according to claim 12, wherein said solid support is beads, gels, ion exchange resin, reverse phase resin, silica matrix or a membrane.

14. The process according to claim 12, wherein the labeled nucleic acid fragment is eluted from the solid support by using a buffer containing betaine.

09736151-121500

SUB
C3

15. The process according to claim 7, wherein the treating step precipitates the labeled nucleic acid fragment at ambient temperature from a solution that contains betaine, DTAB and unlabeled nucleic acid.

5 16. The process according to claim 7, wherein the treating step dilutes an *in vitro* nucleic acid amplification mixture.

17. The process according to claim 1, wherein the fragmenting and attaching steps are performed in a single reaction mixture.

18. The process according to claim 4, wherein the fragmenting and attaching steps are performed in a single reaction mixture.

10 19. The process according to claim 1, wherein the fragmenting and attaching steps are effected in separate steps.

20. The process according to claim 4, wherein the fragmenting and attaching steps are effected in separate steps.

15 21. The processing according to claim 4, wherein the nucleic acid is DNA, RNA, a chimeric DNA-RNA polymer, DNA comprising at least one thiophosphate nucleotide or RNA comprising at least one thiophosphate nucleotide.

22. The process according to claim 1, wherein the attaching step attaches a label to an internal or terminal thiophosphate or to an internal or terminal phosphate of said fragment.

20 23. The process according to claim 1, wherein the fragmenting step further includes use of a chemical catalyst.

25 24. The process according to claim 23, wherein the chemical catalyst is a base selected from the group consisting of imidazole, a substituted analogue of imidazole, and a compound that includes an imidazole ring or substituted analogue of an imidazole ring.

09736151-121500

25. The process according to claim 23, wherein the chemical catalyst is selected from the group consisting of N-methylimidazole, MOPS, HEPES, PIPES, and bioorganic polyamines.

26. A process of fragmenting and labeling a synthetic or natural nucleic acid, comprising the steps of:

chemically fragmenting the nucleic acid in the presence of at least one multivalent metal cation selected from the group consisting of Ba^{2+} , Be^{2+} , Cd^{2+} , Ce^{3+} , Cr^{3+} , Eu^{3+} , Fe^{2+} , In^{3+} , Lu^{3+} , Ni^{2+} , Pb^{2+} , Ru^{3+} , Sr^{2+} , Tb^{3+} , Tm^{3+} and Yb^{3+} in a substantially aqueous solution to produce a plurality of fragments; and

attaching at least one label to at least one of said fragments with a labeling agent to product a detectably labeled fragment in said aqueous solution.

27. The processing according to claim 26, wherein the nucleic acid is DNA, RNA, a chimeric DNA-RNA polymer, DNA comprising at least one thiophosphate nucleotide or RNA comprising at least one thiophosphate nucleotide.

28. The process according to claim 26, wherein the nucleic acid is an RNA or RNA comprising at least one thiophosphate nucleotide, and the multivalent metal cation is Sr^{2+} , Ba^{2+} , Pb^{2+} , Cd^{2+} , Fe^{2+} , Ni^{2+} , Ru^{3+} , Ce^{3+} , Eu^{3+} , Tb^{3+} , Tm^{3+} , Yb^{3+} or Lu^{3+} , and the fragmenting step further includes use of a chemical catalyst.

29. The process according to claim 26, wherein the nucleic acid is a DNA and the multivalent metal cation is Tb^{3+} , and the fragmenting step further includes use of a chemical catalyst.

30. The process according to claim 26, wherein the nucleic acid is RNA or RNA comprising at least one thiophosphate nucleotide, and the multivalent metal cation is Cr^{3+} , Ce^{3+} , Yb^{3+} , Tb^{3+} , Eu^{2+} or Pb^{2+} .

SUB
C4

09736451.121500

31. The process according to claim 26, wherein the nucleic acid is DNA or DNA comprising at least one thiophosphate nucleotide, and the multivalent metal cation is Be^{2+} , Cr^{3+} , Pb^{2+} , In^{3+} , Tb^{3+} , Ce^{3+} , Yb^{3+} or Ni^{2+} .

32. The process according to claim 26, wherein the multivalent metal cation is Tb^{3+} or Ce^{3+} .

33. The process according to claim 17, wherein the mixture contains the labeling agent in a concentration of between 0.1 mM to 4 mM.

34. The process according to claim 33, wherein the mixture contains the labeling agent in a concentration of between 0.1 mM to 1 mM.

35. The process according to claim 33, wherein the labeling agent concentration is between 0.3 mM to 0.55 mM.

36. The process according to claim 17, wherein the labeling agent contains alkyl halide or haloacetamide reactive functions.

37. The process according to claim 1, wherein the labeling agent is 5-(bromomethyl)fluorescein, 6-(bromomethyl)fluorescein, 6-iodoacetamidofluorescein or 5-iodoacetamidofluorescein.

ADD
C5

005221-13500